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(54) NOVEL VEGF-LIKE FACTORS

(57) A novel human gene having a significant homology with a VEGF-C gene, a member of the VEGF family, has been isolated by the PCR method using primers designed based on the sequence of EST that is assumed to be homologous with the C-termial region of the VEGF-C gene. Mouse and rat genes have been isolated based on the human gene isolated as above. A protein encoded by the above human gene has been isolated by introducing the gene into *Escherichia coli* and expressing it. The isolated protein and genes can be applied to, for example, gene therapy for the VEGF-D deficiency, wound healing, and promotion of collateral vessel formation. Furthermore, VEGF-D protein inhibitors can be used as a novel anticancer drug, etc.

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Description

Technical Field

[0001] The present invention relates to a protein factor involved in angiogenesis in humans and falls in the field of genetic engineering.

Background Art

[0002] The process of angiogenesis, in which endothelial cells existing in the inner wall of blood vessels of animals generate new blood vessels, is triggered by transduction of a specific signal. A variety of substances are reportedly involved in this signal transduction. The most notable substance among them is the vascular endothelial growth factor (VEGF). VEGF is a protein factor which was isolated and purified, and can increase the proliferation of endothelial cells and the permeability of blood vessels (Senger, D. R. et al., Science 219: 983-985 (1983); Ferrara, N. and Henzel, W. J., Biochem. Biophys. Res. Commun. 161: 851-858 (1989)). It has been reported that the human VEGF gene contains eight exons and produces four subtypes consisting of 121, 165, 189, or 206 amino acid residues, depending on the difference in splicing, which causes different secretionpatterns (Houck, K. A. et al., Mol. Endocrinol. 5: 1806-1814 (1991)). It has also been reported that there is a VEGF-specific receptor, flt-1, and that the binding of VEGF to flt-1 is important for the signal transduction (Vries, C. D. et al., Science 255: 989-991 (1992)).

[0003] Placental growth factor (PIGF) and platelet-derived growth factor (PDGF) have thus far been isolated and are factors related to VEGF. These factors are found to promote proliferation activities of vascular endothelial cells (Maglione, D. et al., Proc. Natl. Acad. Sci. USA 88: 9267-9271 (1991); Betsholtz, C. et al., Nature 320: 695-699 (1986)). In addition, VEGF-B (Olofsson, B. et al., Proc. Natl. Acad. Sci. USA 93: 2576-2581 (1996)) and VEGF-C (Lee, J. et al.,Proc. Natl. Acad. Sci. USA 93: 1988-1992 (1996);Joukov, V. et al., EMBO J. 15, 290-299 (1996)) have recently been isolated.

[0004] These factors appear to constitute a family, and this may contain additional unknown factors.

[0005] It has been suggested that VEGF is involved in not only vascular formation at the developmental stage but also in the pathological neovascularization associated with diabetes, rheumatoid arthritis, retinopathy, and the growth of solid tumors. Furthermore, in addition to its vascular endothelial cell growth-promoting effects listed above, VEGF's ability to increase vascular permeability was suggested to be involved in the edema formation resulting from various causes. Also, these VEGF family factors may act on not only the blood vessels but also the blood cells and the lymphatic vessels. They may thus play a role in the differentiation and proliferation of blood cells and the formation of lymphatic vessels. Consequently, the VEGF family factors are presently drawing extraordinary attention for developing useful, novel drugs.

Disclosure of the Invention

[0006] An objective of the present invention is to isolate a novel protein belonging to the VEGF family and a gene encoding the protein. We searched for genes having homology to VEGF-C, which is a recently cloned VEGF family gene, against Expressed Sequence Tags (EST) and Sequence Tagged Sites (STS) in the GenBank database. As a result, we found an EST that was assumed to have homology to the C-terminal portion of VEGF-C. We then designed primers based on the sequence, and amplified and isolated the corresponding cDNA using the 5' RACE method and the 3' RACE method. The nucleotide sequence of the isolated cDNA was determined, and the deduced amino acid sequence therefrom revealed that the amino acid sequence had significant homology to that of VEGF-C. Based on the homology, we have assumed that the isolated human clone is a fourth member of the VEGF family (hereinafter designated as VEGF-D). We have also succeeded in expressing the protein encoded by the isolated human VEGF-D gene in E. coli cells, and have also purified and isolated it. Furthermore, we have succeeded in isolating the mouse and rat VEGF-D genes using the isolated human VEGF-D gene.

[0007] In particular, the present invention relates to a novel protein belonging to the VEGF family and a gene encoding the protein. More specifically it relates to

(1) A protein shown by SEQ ID NO.1 or having the amino acid sequence derived therefrom in which one or more amino acids are substituted, deleted, or added;
(2) A protein encoded by a DNA that hybridizes with the DNA shown by SEQ ID NO. 2;
(3) A DNA encoding the protein of (1);
(4) A DNA hybridizing with the DNA shown by SEQ ID NO. 2;
(5) A vector containing the DNA of (3) or (4);
(6) A transformant carrying the vector of (5);

(7) A method of producing the protein of (1) or (2), which comprises culturing the transformant of (6);

(8) An antibody binding to the protein of (1) or (2);

(9) A method of screening a compound binding to the protein of (1) or (2), which comprises a step of detecting the activity of the protein of (1) or (2) to bind to a test sample; and

(10) A compound binding to the protein of (1) or (2), wherein said compound has been isolated by the method of (9).

[0008] The protein of the present invention (VEGF-D) has significant homology to VEGF-C and can be considered to be a fourth factor of the VEGF family. Since the major function of VEGF is vascular formation at the developmental stage and VEGF is considered to be involved in the pathological neovascularization associated with diabetes, rheumatoid arthritis, retinopathy, and the growth of solid tumors, the protein of the present invention is thought to have similar functions.

[0009] A person skilled in the art could prepare functionally equivalent proteins through modifying VEGF-D of the present invention by adding, deleting, or substituting one or more of the amino acids of VEGF-D shown by SEQ ID NO. 1 using known methods. Modifications of the protein can also occur naturally in addition to the artificial modifications described above. These modified proteins are also included in the present invention. Known methods for adding, deleting, or substituting amino acids include the overlap extension polymerase chain reaction (OE-PCR) method (Gene, 1989, 77 (1): 51).

[0010] The DNA encoding VEGF-D of the present invention, shown by SEQ ID NO. 2, is useful for isolating DNAs encoding the proteins having similar functions to VEGF-D in other organisms. For example, a person skilled in the art could routinely isolate homologs of human VEGF-D of the present invention from other organisms by allowing the DNA shown by SEQ ID NO. 2, or part thereof, as a probe, to hybridize with the DNA derived from other organisms. The DNA that hybridizes with the DNA shown by SEQ ID NO. 2 is also included in the present invention. The other organisms include mice, rats, and rabbits.

[0011] The DNA encoding a protein that is functionally equivalent to VEGF-D usually has high homology to the DNA shown by SEQ ID NO. 2. The high homology used herein means at least 70% or higher, more preferably 80% or higher, and still more preferably 90% or higher of sequence homology.

[0012] An example of the hybridization conditions for isolating the DNA having high homology will be given below. Prehybridization is performed in ExpressHyb Solution at 68°C for 30 minutes. The probe labeled with a radioisotope is denatured at 95°C to 100°C for 2 to 5 minutes and rapidly chilled on ice. The probe is added to a new ExpressHyb Solution. The blot is transferred to the solution containing the probe and allowed to hybridize under a temperature gradient of 68°C to 55°C for 2 hours. The blot is washed four times, for 10 minute each, with a 2 x SSC solution containing 0.05% SDS at room temperature. The blot is then washed with a 0.1 x SSC solution containing 0.1% SDS at 45°C for 3 minutes. The blot is subjected to autoradiography.

[0013] An example of the hybridization conditions for isolating the DNA having very high homology will be given below. Prehybridization is performed in ExpressHyb Solution at 68°C or 30 minutes. The probe labeled with a radioisotope is denatured at 95°C to 100°C for 2 to 5 minutes and rapidly chilled on ice. The probe is added into a new ExpressHyb Solution. The blot is transferred into the solution containing the probe, and allowed to hybridize at 68°C for 1 hour. The blot was washed four times, for 10 minute each, with a 2 x SSC solution containing 0.05% SDS at room temperature. The blot was then washed with a 0.1 X SSC solution containing 0.1% SDS at 50°C for 40 minutes, during which the solution was replaced once. The blot was then subjected to autoradiography.

[0014] Note that the hybridization condition can vary depending on the length of the probe (whether it is an oligomer or a probe with more than several hundred bases), the labeling method (whether the probe is radioisotopically labeled or non-radioisotopically labeled), and the type of the target gene to be cloned. A person skilled in the art would properly select the suitable hybridization conditions. In the present invention, it is especially desirable that the condition does not allow the probe to hybridize with the DNA encoding VEGF-C.

[0015] The DNA of the present invention is also used to produce VEGF-D of the present invention as a recombinant protein. Specifically, the recombinant protein can be produced in large quantity by incorporating the DNA encoding VEGF-D (for example, the DNA shown by SEQ ID NO. 2) into a suitable expression vector, introducing the resulting vector into a host, and culturing the transformant to allow the recombinant protein to be expressed.

[0016] The vector to be used for producing the recombinant protein is not particularly restricted. However, vectors such as pGEMEX-1 (Promega) or pEF-BOS (Nucleic Acids Res. 1990, 18(17): p.5322) are preferable.. Suitable examples of the host into which the vector is introduced include E. coli cells, CHO cells, and COS cells.

[0017] The VEGF-D protein expressed by the transformant can be purified by suitably combining purification treatments such as solubilization with a homogenizer or a sonicator, extraction by various buffers, solubilization or precipitation by acid or alkali, extraction or precipitation with organic solvents, salting out by ammonium sulfate and other agents, dialysis, ultrafiltration using membrane filters, gel filtration, ion exchange chromatography, reversed-phase chromatography, counter-current distribution chromatography, high-performance liquid chromatography, isoelectric

focusing, gel electrophoresis, or affinity chromatography in which antibodies or receptors are immobilized.

[0018] Once the recombinant protein is obtained, antibodies against it can be prepared using known methods. The known methods include preparing polyclonal antibodies by immunizing rabbits, sheep, or other animals with the purified protein, and preparing monoclonal antibodies from the antibody-producing cells of immunized mice or rats. These antibodies will make it possible to quantify VEGF. Although the antibodies thus obtained can be used as they are, it will be more effective to use the humanized antibodies to reduce the immunogenicity. The methods of humanizing the antibodies include the CDR graft method and the method of directly producing a human antibody. In the CDR Graft method, the antibody gene is cloned from the monoclonal antibody-producing cells and its antigenic determinant portion is transplanted into an existing human antibody. In the method of directly producing a human antibody, a mouse whose immune system has been replaced by the human immune system is immunized, similar to ordinary monoclonal antibodies. The VEGF-D protein or its antibody thus obtained can be administered into the body by subcutaneous injection or a similar method.

[0019] A person skilled in the art could screen compounds that bind to the protein of the present invention by known methods.

[0020] For example, such compounds can be obtained by making a cDNA library on a phage vector (such as λgt11 and ZAP) from the cells expected to express the protein that binds to the protein of the present invention (such as lung, small intestine, and heart cells of mammals), expressing the cDNAs on LB-agarose, fixing the expressed proteins onto a filter, preparing the purified protein of the present invention as a biotin-labeled or a fusion protein with the GST protein, and reacting this protein with the above filter. The desired compounds could then be detected by west western blotting using streptavidin or an anti-GST antibody (Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A., and Schlessinger, J. (1991) Cloning of P13 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases, Cell 65: 83-90). Another method comprises the following steps. First, express the protein of the present invention fused with the SRF binding domain or the GAL4 binding domain in yeast cells. Second, prepare a cDNA library which expresses cDNAs fused with the transcription activation domain of VP16 or GAL4 from the cells expected to express a protein that binds to the protein of the present invention. Third, introduce the cDNA into the above yeast cells. Fourth, isolate the library-derived cDNA from the positive clones. Finally, introduce the isolated cDNA into E. coli to allow it to be expressed. (When a protein that binds to the protein of the present invention is expressed in yeast cells, the reporter gene is activated and the positive clone can be detected.) This method can be performed using the two-hybrid system (MATCHMAKER Two-Hybrid system, Mammalian MATCHMAKER Two-Hybrid Assay Kit, or MATCHMAKER One-Hybrid System (all by Clontech) orthe HybriZAP Two-Hybrid Vector System (Stratagene) (Dalton, S. and Treisman, R. (1992) Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element, Cell 68: 597-612). Alternatively, the binding proteins can be screened by preparing a cDNA library from the cells expected to express a substance, such as a receptor, which binds to the protein of the present invention (for example, vascular endothelial cells, bone marrow cells, or lymph duct cells), introducing it into such cells as COS, detecting the binding of the protein of the present invention by itself or labeled with a radioisotope or a fluorescence, and cloning proteins that bind to the protein of the present invention (Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T., and Kishimoto, T. (1988) Cloning and expression of human interleukin-6 (BSF-2/IFN beta2) receptor, Science 241: 825-828, Fukunaga, R., Ishizaka-Ikeda, E., Seto, Y., and Nagata, S. (1990) Expression cloning of a receptor for murine granulocyte colony-stimulating factor, Cell 61: 341-350). Still another method comprises applying the culture supernatant or the cellular extract of the cells expected to express a protein that binds to the protein of the present invention onto an affinity column to which the protein of the present invention has been immobilized, and purifying the proteins specifically bound to the column. In addition, a DNA encoding the protein that binds to the protein of the present invention can be obtained by determining the amino acid sequence of the binding protein, synthesizing oligonucleotides based on the sequence, and screening a cDNA library with the oligonucleotides as probes.

[0021] Furthermore, compounds that bind to the protein of the present invention can be screened by contacting compounds, a natural substance bank, or a random phage peptide display library with the immobilized protein of the present invention and detecting the molecules bound to the protein. These compounds can also be screened by high throughput screening utilizing combinatorial chemistry technology (Wrighton, N. C., Farrell, F. X., Chang, R., Kashyap, A. K., Barbone, F. P., Mulcahy, L. S., Johnson, D. L., Barrett, R. W., Jolliffe, L. K., and Dower, W. J., Small peptides as potent mimetics of the protein hormone erythropoietin, Science (United States) Jul 26 1996, 273: 458-464, Verdine, G.L., The combinatorial chemistry of nature, Nature (England) Nov 7 1996, 384: 11-13, Hogan, J.C. Jr. Directed combinatorial chemistry, Nature (England) Nov 7 1996, 384: 17-19).

[0022] VEGF-D of the present invention may be used for gene therapy by introducing the VEGF-D gene into the body of the patient with the VEGF-D deficiency, or expressing the gene in the body. An anti-sense DNA of the VEGF-D gene may also be used to inhibit the expression of the gene itself, thereby suppressing the pathological neovascularization.

[0023] Among the many available methods to introduce the VEGF-D gene or its antisense DNA into the body, the retrovirus method, the liposome method, the cationic liposome method, and the adenovirus method are preferable.

[0024] In order to express these genes in the body, the genes can be incorporated into a suitable vector and introduced into the body by the retrovirus method, the liposome method, the cationic liposome method, or the adenovirus method. Although the vectors to be used are not particularly limited, such vectors as pAdexlcw and pZIPneo are preferable.

[0025] The present invention may also be applied for diagnosing disorders caused by abnormalities of the VEGF-D gene, for example, by PCR to detect an abnormality of the nucleotide sequence of the VEGF-D gene.

[0026] Furthermore, according to the present invention, the VEGF-D protein or its agonists can be used to heal wounds, promote collateral vessel formation, and aid hematopoiesis by the hematopoietic stem cells, by taking advantage of the angiogenic effect of the VEGF-D protein. The antibodies against the VEGF-D protein or its antagonists can be used as the therapeutic agents for pathological neovascularization, lymphatic dysplasia, dyshematopoiesis, or edemas arising from various causes. The anti-VEGF-D antibodies can be used for diagnosing diseases resulting from abnormal production of VEGF-D by quantifying VEGF-D.

Brief Description of the Drawings

[0027]

Figure 1 shows the relationship among the VEGF-D gene, the EST sequences, and the primers used for cloning.
Figure 2 compares the amino acid sequences of EST (H24828) and VEGF-C.
Figure 3 compares the amino acid sequences deduced from the VEGF-D gene and from the known genes of the VEGF family proteins.
Figure 4a shows the hydrophobicity plot of VEGF-D. Figure 4b shows the prediction of the cleavage site of the VEGF-D signal peptide.

Best Mode for Implementing the Invention

[0028] The following examples illustrate the present invention in detail, but are not to be construed to limit the scope of the invention.

Example 1. Homology search by TFASTA method

[0029] The sequence CGPNKELDENTCQCVC (SEQ ID NO. 3) was designed based on the consensus sequence found in the BR3P (Balbiani ring 3 protein) repeat at the C-terminus of VEGF-C. The entire ESTs and STS sequences in the Genbank database (as of 29 February 1996) were then searched by the TFASTA method (Person and Lipman, Proc. Natl. Acad. Sci. USA 85: 2444-2448 (1988)). The searching conditions used are shown below (Table 1).

Table 1

Sequences	392,210
Symbols	135,585,305
Word Size	2
Gap creation penalty	12.0
Gap extension penalty	4.0

[0030] As a result, an EST (Accession No. H24828) that is considered to code the consensus sequence was found. The sequence is one of the ESTs registered by The WashU-Merck EST Project, and nine out of 16 amino acid residues were identical. Further searching for UniGene by NCBI based on this sequence revealed that five registered sequences (T64149, H24780, H24633, H24828, and T64277 (as of 1 March 1996)), including the above EST, were considered to be derived from the same gene. T64277 and T64149, as well as H24828 and H24780, are the combination of the 5' sequence and the 3' sequence of the same clones, and the length of the insert in both of these clones was 0.9 kb (Fig. 1).

[0031] Translating the H24828 sequence into a protein sequence in a frame where homology is found suggested that this sequence codes 104 C-terminal amino acid residues. Comparing this amino acid sequence with the C-terminus of VEGF-C, 28 out of 104 amino acids (27%) were identical. Moreover, the amino acids that are important for maintaining the protein structure, such as cysteine and proline, were well conserved (Fig. 2). Conserved sequences are shown in a

black box.

Example 2. cDNA cloning from a library

[0032] Primers for 5' RACE and 3' RACE (5' RACE primer: 5'-AGGGATGGGGAACTTGGAACGCTGAAT-3' (SEQ ID NO. 4), 3' RACE primer: 5'-GATCTAATCCAGCACCCCAAAAACTGC-3'(SEQ ID NO. 5)) were designed (Fig. 1). A double-stranded cDNA was synthesized from human lung-derived polyA$^+$ RNA using reverse transcriptase. PCR was then performed using Marathon-Ready cDNA, Lung (Chlontech), having an adapter cDNA ligated to both ends as a template cDNA, and using the above primer and adapter primer (AP-1 primer) as primers. The above adapter cDNA contains the regions to which the adapter primers AP-1 and AP-2 hybridize. The PCR was performed in a manner such that the system was exposed to treatment at 94°C for 1 min; five cycles of treatment at 94°C for 30 sec and at 72°C for 4 min; five cycles of treatment at 94°C for 30 sec and at 70°C for 4 min; then 25 cycles of treatment at 94°C for 20 sec and at 68°C for 4 min. (TaKaRa Ex Taq (Takara Shuzo) and the attached buffer were used as Taq polymerase instead of Advantage KlenTaq Polymerase Mix.) As a result, 1.5kb fragments were amplified at the 5' region and 0.9kb fragments at the 3' region. These fragments were cloned with the pCR-Direct Cloning System (Clontech), CR-TRAP Cloning System (GenHunter), and PT7Blue-T vector (Novagen). When the 5'-RACE fragment was cloned into the pCR-Direct vector, the fragment was amplified again using 5'-CTGGTTCGGCCCAGAACTTGGAACGCTGAATCA-3' (SEQ No. 7) and 5'-CTCGCTCGCCCACTAATACGACTCACTATAGG-3' (SEQ ID NO. 8) as primers.

Example 3. Nucleotide sequence analysis

[0033] ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq DNA Polymerase FS and 377 A DNA Sequencer (ABI) were used for DNA sequencing. The primers used are the primers in the vectors (5'-AATTAACCCTCACTAAAGGG-3' (SEQ ID NO. 9), 5'-CCAGGGTTTTCCCAGTCACGAC-3'(SEQ ID NO. 10)), AP-2 primer (5'-ACTCACTATAGGGCTCGAGCGGC-3' (SEQ ID NO. 11)), and 10 primers in the sequence shown below (Table 2).

Table 2

SQ1 (SEQ ID NO. 12)	5'-AAGTCTGGAGACCTGCT-3'
SQ2 (SEQ ID NO. 13)	5'-CAGCAGGTCTCCAGACT-3'
SQ3 (SEQ ID NO. 14)	5'-CGCACCCAAGGAATGGA-3'
SQ4 (SEQ ID NO. 15)	5'-TGACACCTGGCCATTCCA-3'
SQ5 (SEQ ID NO. 16)	5'-CATCAGATGGTAGTTCAT-3'
SQ6 (SEQ ID NO. 17)	5'-ATGCTGAGCGAGAGTCCATA-3'
SQ7 (SEQ ID NO. 18)	5'-CACTAGGTTTGCGGCAACTT-3'
SQ8 (SEQ ID NO. 19)	5'-GCTGTTGGCAAGCACTTACA-3'
SQ9 (SEQ ID NO. 20)	5'-GATCCATCCAGATCCCTGAA-3'
SQ10 (SEQ ID NO. 21)	5'-CAGATCAGGGCTGCTTCTA-3'

[0034] Determining the nucleotide sequence of the 1.5kb fragment at the 5'-side and the 0.9kb fragment at the 3'-side revealed that the sequence of the overlapping region was identical, confirming that 5'- and 3'-side cDNAs of the desired gene were obtained. Determining the entire nucleotide sequence of the cDNA revealed that this novel gene has the full length of 2 kb and can code a protein consisting of 354 amino acid residues (SEQ ID NO. 1 and SEQ ID NO. 2). Figure 1 shows the relation between this gene and the EST sequences registered in the Genbank database. Comparing the amino acid sequence with other VEGF family proteins revealed that the amino acids that are well conserved between family proteins are also conserved in this novel gene, and therefore this gene is obviously a new member of the VEGF family (Fig. 3). In Fig. 3, HSVEGF indicates human VEGF; HSVEGF-D, HSVEGF-C, and HSVEGF-B indicate human VEGF homologues (human VEGF-D, human VEGF-C, and human YEGF-B, respectively); HSPDGF-A indicates human PDGF-A; HSPDGF-B indicates human PDGF-B; and HSP1GF2 indicates human P1GF2. The conserved sequences are shown in a black box. Since VEGF-D is highly homologous to VEGF-C that was cloned as the Flt4 ligand, it was presumed to be a ligand to a Flt-4-like receptor.

[0035] Deducing the signal peptide cleavage site (Fig. 4b) by hydrophobicity plot (Fig. 4a) and the method of von Heijne (von Heijne, G, Nucleic Acids Res. 14, 4683-4690(1986)), N-terminal 21 amino acid residues may be cleaved as

signal peptides, and they may also undergo additional processing like VEGF-C.

Example 4. Northern blot analysis

[0036] A 1kb fragment, which had been cut out by digestion with EcoRI from the 5'-fragment subcloned into pCR-Direct vector, was labeled with[α-^{32}P]dCTP and used as a probe. Labeling was performed by random priming using Ready-to Go DNA labeling beads (Pharmacia). Hybridization was performed in ExpressHyb Hybridization Solution (Clontech) by the usual method using Multiple Tissue Northern (MTN) Blot-Human, Human II, Human Fetal, and Human Cell lines (Clontech). Significant expression was observed in lung, heart, and intestine. Weak expression was observed in skeletal muscle, ovary, colon, and pancreas. The apparent molecular weight of the mRNA was 2.2 kb, and the cloned fragment seemed to be almost the full length of the gene.

Example 5. VEGF-D protein expression in E. coli

[0037] Two primers, 5'-TCCAGATCTTTTGCGGCAACTTTCTATGACAT-3' (SEQ ID NO. 22) and 5'-CAGGTCGACT-CAAACAGGCACTAATTCAGGTAC-3' (SEQ ID NO. 23), were synthesized to amplify the region corresponding to the 89th to 181st amino acid residues of human VEGF cDNA. The thus-obtained DNA fragment was digested with restriction enzymes BglII and SalI, and ligated using ligation kit II (Takara Shuzo Co., Ltd) to plasmid pQE42 ((QIAGEN), which had been digested with restriction enzymes BamHI and SalI. The resulting plasmid was introduced into E. coli SG19003[pREP4] (QIAGEN),and a plasmid, which was obtained as designed without any mutation, was selected (pQE42-BS3). Plasmid pQE42-BS3 was introduced into E. coli BL21 (Invitorogen) and cultured in 10 ml of L Broth containing 100 mg/l bicucilline (ampicillin sodium for injection, Meiji Seika Kaisha, Ltd.). 200 ml of fresh L Broth was then inoculated with the culture. After incubation at 37°C for 1.5 hours, IPTG was added to 3 mM, and the culture was further incubated at 37°C for 5 hours. After cells were harvested, a protein was purified with a Ni-NTA column following the protocol of QIAexpress TypeII kit.

Example 6. Expression of DHFR-VEGF-D fusion protein in E. coli

[0038] The region corresponding to the 89th to 181st amino acid residues of human VEGF cDNA was amplified with the same primers used in Example 5. The thus-obtained DNA fragment was digested with restriction enzymes BglI and SalI. The fragment was then ligated using ligation kit II (Takara Shuzo Co., Ltd.) to the plasmid pQE40 (QIAGEN), which had been digested with restriction enzymes BamHI and SalI. The resulting plasmid was introduced into E. coli SG19003[pREP4] (QIAGEN), and a plasmid, which was obtained as designed without any mutation, was selected (pQE40-BS3). Plasmid pQE40-BS3 was introduced into E. coli BL21 (Invitrogen) and cultured in 10 ml of L Broth containing 100 mg/l bicucilline (ampicillin sodium for injection, Meiji Seika Kaisha, Ltd.). 200 ml of fresh L Broth was then inoculated with the culture. After incubation at 37°C for 1.5 hours, IPTG was added to 3mM, and the culture was further incubated at 37°C for 5 hours. After cells were harvested, a DHFR-VEGF-D fusion protein was purified with a Ni-NTA column following the protocol of a QIAexpress TypeII kit.

Example 7. Cloning mouse VEGF-D cDNA

[0039] Two Hybond-N+ (Amersham) filters (20 cm x 22 cm) on which 1.5 x 10^5 pfu of Mouse lung 5'-stretch cDNA library was transferred were prepared. Gradient hybridization from 68°C to 55°C was performed for 2 hours in ExpressHyb Hybridization Solution (Clontech) using as a probe an approximately 50 ng Pvu II fragment of human VEGF-D, which had been labeled with α^{32}P-dCTP (Amersham) using Ready-To-Go DNA Labeling Beads(-dCTP) (Pharmacia). The filters were washed four times in 2 x SSC, 0.05% SDS at room temperature for 10 min, then washed in 0.1 x SSC, 0.1% SDS at 45°C for 3 min. The washed filters were exposed overnight at -80°C using HyperFilm MP (Amersham) and intensifying paper. Positive clones were subjected to the second screening in the same manner as above to isolate a single clone. Isolated lambda DNAs were purified from the plate lysate using a QIAGEN Lambda MAX I Kit (Qiagen). Insert DNAs were cut out with EcoRI and subcloned into pUC118 EcoRI/BAP (Takara Shuzo Co., Ltd.). Its nucleotide sequence was then determined with ABI377 sequencer (Perkin Elmer). The cDNA coding the full length of mouse VRGF-D was reconstructed with two of the obtained clones that overlapped each other. SEQ ID NO. 24 shows the nucleotide sequence of mouse VEGF-D cDNA and the deduced amino acid sequence therefrom.

Example 8. Cloning rat VEGF-D cDNA

[0040] Two Hybond-N+ (Amersham) filters (20 cm x 22 cm), on which 1.5 x 10^5 pfu of Rat lung 5'-stretch cDNA library had been transferred, were prepared. Gradient hybridization from 68°C to 55°C was performed for 2 hours in

ExpressH.Fyb Hybridization Solution (Clontech) using as a probe an approximately 1 μg fragment containing 1-782 bp of the mouse VEGF-D cDNA which had been labeled with α^{32}P-dCTP (Amersham) using Ready-To-Go DNA Labeling Beads(-dCTP) (Pharmacia). The filters were washed four times in 2 x SSC, 0.05% SDS at room temperature for 10 min, then washed in 0.1 x SSC, 0.1% SDS at 45°C for 3 min. The washed filters were exposed overnight at -80°C using HyperFilm MP (Amersham) and intensifying paper. Positive clones were subjected to the second screening in the same manner as above to isolate a single clone. The isolated positive clone was excised into pBluescript using E. coli SOLAR (Stratagene) and helper phage ExAssist (Stratagene), then the sequence was determined with ABI377 sequencer (Perkin Elmer). The sequence seemed to be the rat VEGF-D cDNA but did not contain the termination codon.

[0041] To obtain the C-terminal cDNA which had not been obtained, PCR was performed using Marathon-Ready rat kidney cDNA (Clontech) as a template and 5' primerGCTGCGAGTGTGTCTGTAAA (SEQ ID NO. 26) and 3' primer GGGTAGTGGGCAACAGTGACAGCAA (SEQ ID NO. 27) with 40 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72 °C for 2 min. After the thus-obtained fragment was subcloned into pGEM-T vector (promega), the nucleotide sequence was determined with ABI377 sequencer (Perkin Elmer). The resulting clone contained the C-terminus of rat VEGF-D. Based on the results of sequencing the clone obtained by plaque hybridization and the clone obtained by PCR, the full length of the rat VEGF-D sequence was determined. SEQ ID NO. 25 shows the determined nucleotide sequence and the deduced amino acid sequence therefrom.

Industrial Applicability

[0042] In the present invention, a novel protein (VEGF-D) having significant homology to VEGF-C and its gene have been isolated. VEGF-D appears to be involved in the pathological neovascularization associated with diabetes, rheumatoid arthritis, the growth of solid tumors, differentiation and proliferation of blood cells, formation of lymphatic vessels, and formation of edema resulting from various causes as well as the normal neovascularization at the developmental stage. The gene of the present invention can be used to diagnos disorders caused by abnormalities of the VEGF-D gene and gene therapy for the VEGF-D deficiency. The VEGF-D protein, which is obtained by expressing the gene of the present invention, can be used for healing wounds, promoting collateral vessel formation, and aiding hematopoietic stemcell proliferation. The antibodies or inhibitors against the VEGF-D protein can be used for treating angiodysplasia and lymphangiodysplasia associated with inflammation, edemas arising from various causes, dyshematopoiesis, and, as a novel anticancer agent, for treating pathological neovascularization. The VEGF-D protein and its antibodies can be useful for diagnosing diseases resulting from abnormal production of VEGF-D.

Sequence Listing

(1) Name or appellation of Applicant: Chugai Research Institute for Molecular Medicine, Inc.

(2) Title of the Invention: Novel VEGF-like Factor

(3) Reference Number: C1-802PCT

(4) Application Number:

(5) Filing date:

(6) Country where the priority application was filed and the application number of the application: Japan, No. Hei 8-185216

(7) Priority date: July 15, 1996

(8) Number of sequences: 27

SEQ ID NO: 1

SEQUENCE LENGTH: 354

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

TISSUE TYPE: lung

SEQUENCE DESCRIPTION:

```
Met Tyr Arg Glu Trp Val Val Val Asn Val Phe Met Met Leu Tyr Val
1               5                   10                  15
Gln Leu Val Gln Gly Ser Ser Asn Glu His Gly Pro Val Lys Arg Ser
            20                  25                  30
Ser Gln Ser Thr Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser
        35                  40                  45
Ser Leu Glu Glu Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu
    50                  55                  60
Trp Arg Cys Arg Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg
65                  70                  75                  80
Ser Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile
                85                  90                  95
Glu Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser
            100                 105                 110
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Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr
115 120 125

Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly
130 135 140

Cys Cys Asn Glu Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr
145 150 155 160

Ile Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro
165 170 175

Glu Leu Val Pro Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu
180 185 190

Pro Thr Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln
195 200 205

Ile Pro Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile
210 215 220

Asp Met Leu Trp Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu
225 230 235 240

Asn Pro Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala
245 250 255

Leu Cys Gly Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val
260 265 270

Cys Lys Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys
275 280 285

Ser Cys Phe Glu Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His
290 295 300

Lys Leu Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe
305 310 315 320

His Thr Arg Pro Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys
325 330 335

Arg Phe Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys
340 345 350

Asn Pro

SEQ ID NO: 2

SEQUENCE LENGTH: 2004

SEQUENCE TYPE: nucleic acid

STRANDEDNESSS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

TISSUE TYPE: lung

FEATURE:

NAME/KEY: CDS

LOCATION: 403..1464

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION:

```
CCAGCTTTCT GTARCTGTAA GCATTGGTGG CCACACCACC TCCTTACAAA GCAACTAGAA     60
CCTGCGGCAT ACATTGGAGA GATTTTTTTA ATTTTCTGGA CAYGAAGTAA ATTTAGAGTG    120
CTTTCYAATT TCAGGTAGAA GACATGTCCA CCTTCTGATT ATTTTTGGAG AACATTTTGA    180
TTTTTTTCAT CTCTCTCTCC CCACCCCTAA GATTGTGCAA AAAAAGCGTA CCTTGCCTAA    240
TTGAAATAAT TTCATTGGAT TTTGATCAGA ACTGATCATT TGGTTTTCTG TGTGAAGTTT    300
TGAGGTTTCA AACTTTCCTT CTGGAGAATG CCTTTTGAAA CAATTTTCTC TAGCTGCCTG    360
ATGTCAACTG CTTAGTAATC AGTGGATATT GAAATATTCA AA ATG TAC AGA GAG       414
                                               Met Tyr Arg Glu
                                                1
TGG GTA GTG GTG AAT GTT TTC ATG ATG TTG TAC GTC CAG CTG GTG CAG      462
Trp Val Val Val Asn Val Phe Met Met Leu Tyr Val Gln Leu Val Gln
5                   10                  15                  20
GGC TCC AGT AAT GAA CAT GGA CCA GTG AAG CGA TCA TCT CAG TCC ACA      510
Gly Ser Ser Asn Glu His Gly Pro Val Lys Arg Ser Ser Gln Ser Thr
                25                  30                  35
TTG GAA CGA TCT GAA CAG CAG ATC AGG GCT GCT TCT AGT TTG GAG GAA      558
Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Ser Leu Glu Glu
            40                  45                  50
CTA CTT CGA ATT ACT CAC TCT GAG GAC TGG AAG CTG TGG AGA TGC AGG      606
Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu Trp Arg Cys Arg
        55                  60                  65
CTG AGG CTC AAA AGT TTT ACC AGT ATG GAC TCT CGC TCA GCA TCC CAT      654
Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg Ser Ala Ser His
    70                  75                  80
CGG TCC ACT AGG TTT GCG GCA ACT TTC TAT GAC ATT GAA ACA CTA AAA      702
Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile Glu Thr Leu Lys
```

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85                  90                  95                  100
GTT ATA GAT GAA GAA TGG CAA AGA ACT CAG TGC AGC CCT AGA GAA ACG    750
Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro Arg Glu Thr
                105                 110                 115
TGC GTG GAG GTG GCC AGT GAG CTG GGG AAG AGT ACC AAC ACA TTC TTC    798
Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr Asn Thr Phe Phe
            120                 125                 130
AAG CCC CCT TGT GTG AAC GTG TTC CGA TGT GGT GGC TGT TGC AAT GAA    846
Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys Cys Asn Glu
        135                 140                 145
GAG AGC CTT ATC TGT ATG AAC ACC AGC ACC TCG TAC ATT TCC AAA CAG    894
Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr Ile Ser Lys Gln
    150                 155                 160
CTC TTT GAG ATA TCA GTG CCT TTG ACA TCA GTA CCT GAA TTA GTG CCT    942
Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu Leu Val Pro
165                 170                 175                 180
GTT AAA GTT GCC AAT CAT ACA GGT TGT AAG TGC TTG CCA ACA GCC CCC    990
Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu Pro Thr Ala Pro
                185                 190                 195
CGC CAT CCA TAC TCA ATT ATC AGA AGA TCC ATC CAG ATC CCT GAA GAA   1038
Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Ile Pro Glu Glu
            200                 205                 210
GAT CGC TGT TCC CAT TCC AAG AAA CTC TGT CCT ATT GAC ATG CTA TGG   1086
Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile Asp Met Leu Trp
        215                 220                 225
GAT AGC AAC AAA TGT AAA TGT GTT TTG CAG GAG GAA AAT CCA CTT GCT   1134
Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu Asn Pro Leu Ala
    230                 235                 240
GGA ACA GAA GAC CAC TCT CAT CTC CAG GAA CCA GCT CTC TGT GGG CCA   1182
Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala Leu Cys Gly Pro
245                 250                 255                 260
CAC ATG ATG TTT GAC GAA GAT CGT TGC GAG TGT GTC TGT AAA ACA CCA   1230
His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val Cys Lys Thr Pro
                265                 270                 275
TGT CCC AAA GAT CTA ATC CAG CAC CCC AAA AAC TGC AGT TGC TTT GAG   1278
Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys Ser Cys Phe Glu
```

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                280                 285                  290
TGC AAA GAA AGT CTG GAG ACC TGC TGC CAG AAG CAC AAG CTA TTT CAC    1326
Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His Lys Leu Phe His
            295                 300                  305
CCA GAC ACC TGC AGC TGT GAG GAC AGA TGC CCC TTT CAT ACC AGA CCA    1374
Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe His Thr Arg Pro
        310                 315                  320
TGT GCA AGT GGC AAA ACA GCA TGT GCA AAG CAT TGC CGC TTT CCA AAG    1422
Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys Arg Phe Pro Lys
325                 330                  335                 340
GAG AAA AGG GCT GCC CAG GGG CCC CAC AGC CGA AAG AAT CCT            1464
Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys Asn Pro
                    345                  350
TGATTCAGCG TTCCAAGTTC CCCATCCCTG TCATTTTTAA CAGCATGCTG CTTTGCCAAG   1524
TTGCTGTCAC TGTTTTTTTC CCAGGTGTTA AAAAAAAAAT CCATTTTACA CAGCACCACA   1584
GTGAATCCAG ACCAACCTTC CATTCACACC AGCTAAGGAG TCCCTGGTTC ATTGATGGAT   1644
GTCTTCTAGC TGCAGATGCC TCTGCGCACC AAGGAATGGA GAGGAGGGGA CCCATGTAAT   1704
CCTTTTGTTT AGTTTTGTTT TTGTTTTTTG GTGAATGAGA AAGGTGTGCT GGTCATGGAA   1764
TGGCAGGTGT CATATGACTG ATTACTCAGA GCAGATGAGG AAAACTGTAG TCTCTGAGTC   1824
CTTTGCTAAT CGCAACTCTT GTGAATTATT CTGATTCTTT TTTATGCAGA ATTTGATTCG   1884
TATGATCAGT ACTGACTTTC TGATTACTGT CCAGCTTATA GTCTTCCAGT TTAATGAACT   1944
ACCATCTGAT GTTTCATATT TAAGTGTATT TAAAGAAAAT AAACACCATT ATTCAAGTCT   2004
```

SEQ ID NO: 3

SEQUENCE LENGTH: 16

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: peptide

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

TISSUE TYPE: lung

SEQUENCE DESCRIPTION:

```
Cys Gly Pro Asn Lys Glu Leu Asp Glu Asn Thr Cys Gln Cys Val Cys
1               5                   10                  15
```

SEQ ID NO: 4
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
AGGGATGGGG AACTTGGAAC GCTGAAT 27

SEQ ID NO: 5
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GATCTAATCC AGCACCCCAA AAACTGC 27

SEQ ID NO: 6
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CCATCCTAAT ACGACTCACT ATAGGGC 27

SEQ ID NO: 7
SEQUENCE LENGTH: 33
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CTGGTTCGGC CCAGAACTTG GAACGCTGAA TCA 33

SEQ ID NO: 8
SEQUENCE LENGTH: 32
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CTCGCTCGCC CACTAATACG ACTCACTATA GG 32

SEQ ID NO: 9
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
AATTAACCCT CACTAAAGGG 20

SEQ ID NO: 10
SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CCAGGGTTTT CCCAGTCACG AC 22

SEQ ID NO: 11
SEQUENCE LENGTH: 23
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
ACTCACTATA GGGCTCGAGC GGC 23

SEQ ID NO: 12
SEQUENCE LENGTH: 17
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
AAGTCTGGAG ACCTGCT 17

SEQ ID NO: 13
SEQUENCE LENGTH: 17
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CAGCAGGTCT CCAGACT 17

SEQ ID NO: 14
SEQUENCE LENGTH: 17
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CGCACCCAAG GAATGGA 17

SEQ ID NO: 15
SEQUENCE LENGTH: 18
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
TGACACCTGG CCATTCCA 18

SEQ ID NO: 16
SEQUENCE LENGTH: 18
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CATCAGATGG TAGTTCAT 18

SEQ ID NO: 17
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
ATGCTGAGCG AGAGTCCATA 20

SEQ ID NO: 18
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CACTAGGTTT GCGGCAACTT 20

SEQ ID NO: 19
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GCTGTTGGCA AGCACTTACA 20

SEQ ID NO: 20
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GATCCATCCA GATCCCTGAA 20

SEQ ID NO: 21
SEQUENCE LENGTH: 19
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CAGATCAGGG CTGCTTCTA 19

SEQ ID NO: 22
SEQUENCE LENGTH: 32
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
TCCAGATCTT TTGCGGCAAC TTTCTATGAC AT 32

SEQ ID NO: 23
SEQUENCE LENGTH: 33
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CAGGTCGACT CAAACAGGCA CTAATTCAGG TAC 33

SEQ ID NO: 24

SEQUENCE LENGTH: 1581

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE:

ORGANISM: mouse

TISSUE TYPE: lung

FEATURE:

NAME/KEY: CDS

LOCATION: 96..1169

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION:

```
TTCCGGGCTT TGCTGGAGAA TGCCTTTTGC AACACTTTTC AGTAGCTGCC TGGAAACAAC    60
TGCTTAGTCA TCGGTAGACA TTTAAAATAT TCAAA ATG TAT GGA GAA TGG GGA      113
                                       Met Tyr Gly Glu Trp Gly
                                        1               5
ATG GGG AAT ATC CTC ATG ATG TTC CAT GTG TAC TTG GTG CAG GGC TTC     161
Met Gly Asn Ile Leu Met Met Phe His Val Tyr Leu Val Gln Gly Phe
            10                  15                  20
AGG AGC GAA CAT GGA CCA GTG AAG GAT TTT TCT TTT GAG CGA TCA TCC     209
Arg Ser Glu His Gly Pro Val Lys Asp Phe Ser Phe Glu Arg Ser Ser
        25                  30                  35
CGG TCC ATG TTG GAA CGA TCT GAA CAA CAG ATC CGA GCA GCT TCT AGT     257
Arg Ser Met Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Ser
    40                  45                  50
TTG GAG GAG TTG CTG CAA ATC GCG CAC TCT GAG GAC TGG AAG CTG TGG     305
Leu Glu Glu Leu Leu Gln Ile Ala His Ser Glu Asp Trp Lys Leu Trp
 55                  60                  65                  70
CGA TGC CGG TTG AAG CTC AAA AGT CTT GCC AGT ATG GAC TCA CGC TCA     353
Arg Cys Arg Leu Lys Leu Lys Ser Leu Ala Ser Met Asp Ser Arg Ser
                75                  80                  85
GCA TCC CAT CGC TCC ACC AGA TTT GCG GCA ACT TTC TAT GAC ACT GAA     401
Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Thr Glu
            90                  95                 100
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ACA CTA AAA GTT ATA GAT GAA GAA TGG CAG AGG ACC CAA TGC AGC CCT    449
Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro
        105                 110                 115
AGA GAG ACA TGC GTA GAA GTC GCC AGT GAG CTG GGG AAG ACA ACC AAC    497
Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Thr Thr Asn
    120                 125                 130
ACA TTC TTC AAG CCC CCC TGT GTA AAT GTC TTC CGG TGT GGA GGC TGC    545
Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys
135                 140                 145                 150
TGC AAC GAA GAG GGT GTG ATG TGT ATG AAC ACA AGC ACC TCC TAC ATC    593
Cys Asn Glu Glu Gly Val Met Cys Met Asn Thr Ser Thr Ser Tyr Ile
                155                 160                 165
TCC AAA CAG CTC TTT GAG ATA TCA GTG CCT CTG ACA TCA GTG CCC GAG    641
Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu
            170                 175                 180
TTA GTG CCT GTT AAA ATT GCC AAC CAT ACG GGT TGT AAG TGC TTG CCC    689
Leu Val Pro Val Lys Ile Ala Asn His Thr Gly Cys Lys Cys Leu Pro
        185                 190                 195
ACG GGC CCC CGC CAT CCT TAC TCA ATT ATC AGA AGA TCC ATT CAG ACC    737
Thr Gly Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Thr
    200                 205                 210
CCA GAA GAA GAT GAA TGT CCT CAT TCC AAG AAA CTC TGT CCT ATT GAC    785
Pro Glu Glu Asp Glu Cys Pro His Ser Lys Lys Leu Cys Pro Ile Asp
215                 220                 225                 230
ATG CTG TGG GAT AAC ACC AAA TGT AAA TGT GTT TTG CAA GAC GAG ACT    833
Met Leu Trp Asp Asn Thr Lys Cys Lys Cys Val Leu Gln Asp Glu Thr
                235                 240                 245
CCA CTG CCT GGG ACA GAA GAC CAC TCT TAC CTC CAG GAA CCC ACT CTC    881
Pro Leu Pro Gly Thr Glu Asp His Ser Tyr Leu Gln Glu Pro Thr Leu
            250                 255                 260
TGT GGA CCG CAC ATG ACG TTT GAT GAA GAT CGC TGT GAG TGC GTC TGT    929
Cys Gly Pro His Met Thr Phe Asp Glu Asp Arg Cys Glu Cys Val Cys
        265                 270                 275
AAA GCA CCA TGT CCG GGA GAT CTC ATT CAG CAC CCG GAA AAC TGC AGT    977
Lys Ala Pro Cys Pro Gly Asp Leu Ile Gln His Pro Glu Asn Cys Ser
    280                 285                 290
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TGC TTT GAG TGC AAA GAA AGT CTG GAG AGC TGC TGC CAA AAG CAC AAG     1025
Cys Phe Glu Cys Lys Glu Ser Leu Glu Ser Cys Cys Gln Lys His Lys
295                 300                 305                 310
ATT TTT CAC CCA GAC ACC TGC AGC TGT GAG GAC AGA TGT CCT TTT CAC     1073
Ile Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe His
                315                 320                 325
ACC AGA ACA TGT GCA AGT AGA AAG CCA GCC TGT GGA AAG CAC TGG CGC     1121
Thr Arg Thr Cys Ala Ser Arg Lys Pro Ala Cys Gly Lys His Trp Arg
            330                 335                 340
TTT CCA AAG GAG ACA AGG GCC CAG GGA CTC TAC AGC CAG GAG AAC CCT     1169
Phe Pro Lys Glu Thr Arg Ala Gln Gly Leu Tyr Ser Gln Glu Asn Pro
        345                 350                 355
TGATTCAACT TCCTTTCAAG TCCCCCCATC TCTGTCATTT TAAACAGCTC ACTGCTTTGT   1229
CAAGTTGCTG TCACTGTTGC CCACTACCCC TGCCCCCCCC CCCCCCCGCC TCCAGGTGTT   1289
AGAAAAGTTG ATTTGACCTA GTGTCATGGT AAAGCCACAT TTCCATGCAA TGGCGGCTAG   1349
GTGATTCCCC AGTTCACTGA CAAATGACTT GTAGCTTCAA ATGTCTTTGC GCCATCANCA   1409
CTCAAAAAGG AAGGGGTCTG AAGAACCCCT TGTTTGATAA ATAAAAACAG GTGCCTGAAA   1469
CAAAATATTA GGTGCCACTC GATTGGGTCC CTCGGGCTGG CCAAATTCCA AGGGCAATGC   1529
TCCTGAATTT ATTGTGCCCC TTCCTTAATG CGGAATTTCC TTTTGTTTGA TT           1581
```

SEQ ID NO: 25

SEQUENCE LENGTH: 1491

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE:

    ORGANISM: rat

    TISSUE TYPE: lung

FEATURE:

    NAME/KEY: CDS

    LOCATION: 270..1247

    IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION:

```
GCCACCTCTT GATTATTTGT GCAGCGGGAA ACTTTGAAAT AGTTTTCATC TCTTTCTCCC     60
ATACTAAGAT TGTGTGTGGC CGTGGGGGAG TCCTTGACTA ACTCAAGTCA TTTCATTGGA    120
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TTTTGATTAC AACTGATCAT GTGATATTTT TTTCCATGTA AAGTTTTGGG GCTTCAAACT   180
TTGCTTCTGG AGAATGCCTT TTGCAACACT TTTCAGTAGC TGCCTGGAAA CAACTGCTTA   240
GCCATCAGTG GACATTTGAA ATATTCAAA ATG TAT GGA GAG TGG GCC GCA GTG     293
                                Met Tyr Gly Glu Trp Ala Ala Val
                                1               5
AAT ATT CTC ATG ATG TCC TAT GTG TAC CTG GTG CAG GGC TTC AGT ATT     341
Asn Ile Leu Met Met Ser Tyr Val Tyr Leu Val Gln Gly Phe Ser Ile
    10                  15                  20
GAA CAC CGA GCA GTG AAG GAT GTT TCT CTT GAG CGA TCA TCC CGG TCT     389
Glu His Arg Ala Val Lys Asp Val Ser Leu Glu Arg Ser Ser Arg Ser
25                  30                  35                  40
GTG TTG GAA CGT TCT GAA CAA CAG ATC CGC GCG GCT TCT ACT TTG GAA     437
Val Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Thr Leu Glu
                45                  50                  55
GAG TTG CTG CAA GTC GCA CAC TCT GAG GAC TGG AAG CTG TGG CGG TGC     485
Glu Leu Leu Gln Val Ala His Ser Glu Asp Trp Lys Leu Trp Arg Cys
            60                  65                  70
CGG TTG AAG CTT AAA AGT CTT GCC AAT GTG GAC TCG CGC TCA ACA TCC     533
Arg Leu Lys Leu Lys Ser Leu Ala Asn Val Asp Ser Arg Ser Thr Ser
        75                  80                  85
CAT CGC TCC ACC AGA TTT GCG GCA ACT TTC TAT GAT ACT GAA ACA CTA     581
His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Thr Glu Thr Leu
    90                  95                  100
AAA GTT ATA GAT GAA GAA TGG CAG AGG ACC CAA TGC AGC CCT AGA GAG     629
Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro Arg Glu
105                 110                 115                 120
ACA TGC GTA GAA GTC GCC AGT GAG CTG GGG AAG ACA ACC AAC ACA TTT     677
Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Thr Thr Asn Thr Phe
                125                 130                 135
TTC AAG CCC CCT TGT GTA AAT GTC TTC CGG TGT GGA GGA TGC TGC AAT     725
Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys Cys Asn
            140                 145                 150
GAA GAG AGC GTG ATG TGT ATG AAC ACA AGC ACC TCC TAC ATC TCC AAA     773
Glu Glu Ser Val Met Cys Met Asn Thr Ser Thr Ser Tyr Ile Ser Lys
        155                 160                 165
CAG CTC TTT GAG ATA TCA GTG CCT CTG ACA TCA GTG CCC GAG TTA GTG     821
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Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu Leu Val
    170                 175                 180
CCT GTT AAA ATT GCC AAC CAT ACG GGT TGT AAG TGT TTG CCC ACG GGC     869
Pro Val Lys Ile Ala Asn His Thr Gly Cys Lys Cys Leu Pro Thr Gly
185                 190                 195                 200
CCC CGG CAT CCT TAT TCA ATT ATC AGA AGA TCC ATT CAG ATC CCA GAA     917
Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Ile Pro Glu
                205                 210                 215
GAA GAT CAA TGT CCT CAT TCC AAG AAA CTC TGT CCT GTT GAC ATG CTG     965
Glu Asp Gln Cys Pro His Ser Lys Lys Leu Cys Pro Val Asp Met Leu
            220                 225                 230
TGG GAT AAC ACC AAA TGT AAA TGT GTT TTA CAA GAT GAG AAT CCA CTG    1013
Trp Asp Asn Thr Lys Cys Lys Cys Val Leu Gln Asp Glu Asn Pro Leu
        235                 240                 245
CCT GGG ACA GAA GAC CAC TCT TAC CTC CAG GAA CCC GCT CTC TGT GGA    1061
Pro Gly Thr Glu Asp His Ser Tyr Leu Gln Glu Pro Ala Leu Cys Gly
    250                 255                 260
CCA CAC ATG ATG TTT GAT GAA GAT CGC TGC GAG TGT GTC TGT AAA GCA    1109
Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val Cys Lys Ala
265                 270                 275                 280
CCA TGT CCT GGA GAT CTC ATT CAG CAC CCG GAA AAC TGC AGT TGC TTT    1157
Pro Cys Pro Gly Asp Leu Ile Gln His Pro Glu Asn Cys Ser Cys Phe
                285                 290                 295
GAA TGC AAA GAA AGT CTG GAA AGC TGT TGC CAA AAG CAC AAG ATG TTT    1205
Glu Cys Lys Glu Ser Leu Glu Ser Cys Cys Gln Lys His Lys Met Phe
            300                 305                 310
CAC CCT GAC ACC TGC AGA TCA ATG GTC TTT TCA CTG TCC CCT            1247
His Pro Asp Thr Cys Arg Ser Met Val Phe Ser Leu Ser Pro
        315                 320                 325
TAATTTGGTT TACTGGTGAC ATTTAAAGGA CATACTAACC TGATTTATTG GGGCTCTTTT  1307
CTCTCAGGGC CCAAGCACAC TCTTAAAGGA ACACAGACGT TTGGCCTCTA AGAAATACAT  1367
GGAAGTATTA TAGAGTGATG ATTAAATTGT CTTCTTGTTT CAAACAGGGT CTCATGATTA  1427
CAGACCCGTA TTGCCATGCC TGCCGTCATG CTATCATGAG CGGAAAAGAA TCACTGGCAT  1487
TTAA                                                               1491
```

SEQ ID NO: 26
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GCTGCGAGTG TGTCTGTAAA 20

SEQ ID NO: 27
SEQUENCE LENGTH: 25
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GGGTAGTGGG CAACAGTGAC AGCAA 25

Claims

1. A protein shown by SEQ ID NO: 1 or having the amino acid sequence derived therefrom in which one or more amino acids are substituted, deleted, or added.

2. A protein encoded by a DNA hybridizing with the DNA shown by SEQ ID NO: 2.

3. A DNA encoding the protein of Claim 1.

4. A DNA hybridizing with the DNA shown by SEQ ID NO: 2.

5. A vector containing the DNA of Claim 3 or 4.

6. A transformant carrying the vector of Claim 5.

7. A method of producing the protein of Claim 1 or 2, which comprises culturing the transformant of Claim 6.

8. An antibody binding to the protein of Claim 1 or 2.

9. A method of screening a compound binding to the protein of Claim 1 or 2, which comprises a step of detecting the activity of the protein of Claim 1 or 2 to bind to a test sample.

10. A compound binding to the protein of Claim 1 or 2, wherein the compound have been isolated by the method of Claim 9.

Fig. 1

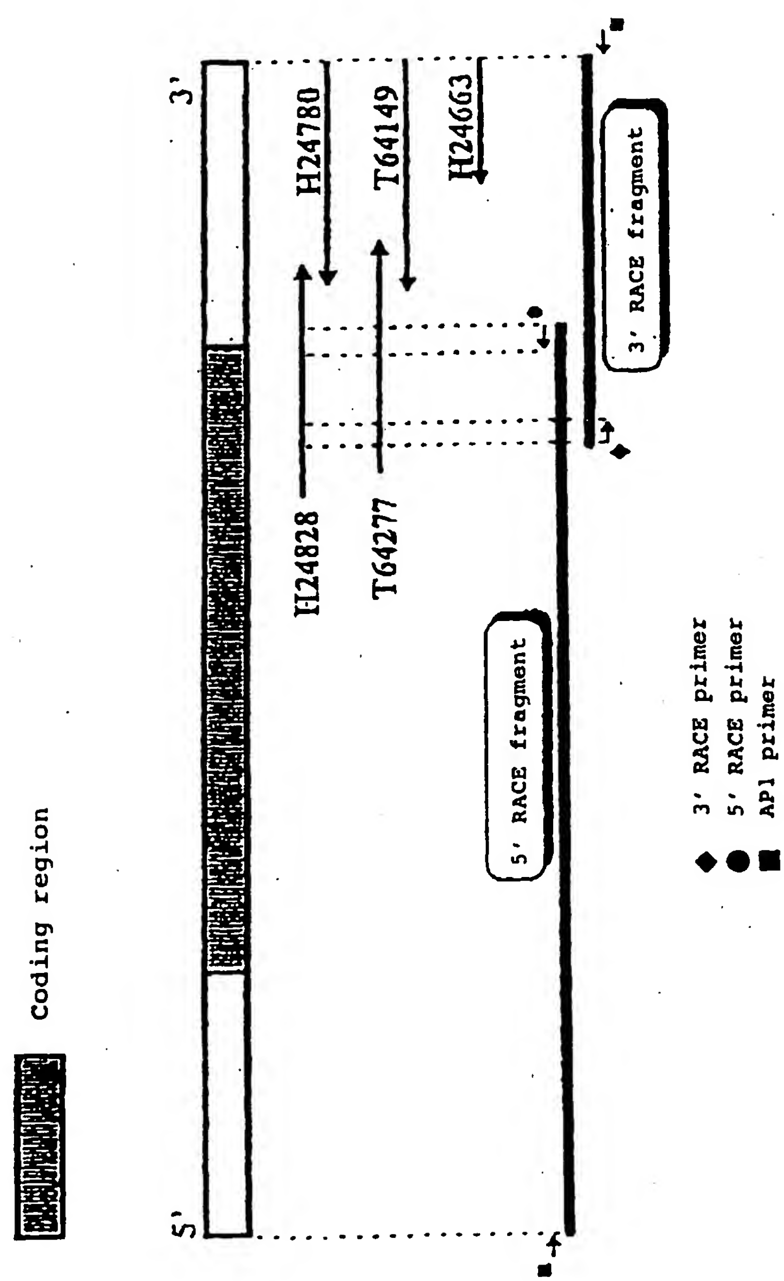

Coding region
3'
5'
H24780
T64149
H24663
H24828
T64277
3' RACE fragment
5' RACE fragment
3' RACE primer
5' RACE primer
AP1 primer

Fig. 2

```
HSVEGFCC*   MHLLGFFSVA CSLLAAALLP GPREAPAAAA AFESGLDLSD AEPDAGEATA    50
H24828      ---------- ---------- ---------- ---------- ----------    50

HSVEGFCC    YASKDLEEQL RSVSSVDELM TVLYPEYWKM YKCQLRKGGW QHNREQANLN   100
H24828      ---------- ---------- ---------- ---------- ----------   100

HSVEGFCC    SRTEETIKFA AAHYNTEILK SIDNEWRKTQ CMPREVCIDV GKEFGVATNT   150
H24828      ---------- ---------- ---------- ---------- ----------   150

HSVEGFCC    FFKPPCVSVY RCGGCCNSEG LQCMNTSTSY LSKTLFEITV PLSQGPKPVT   200
H24828      ---------- ---------- ---------- ---------- ----------   200

HSVEGFCC    ISFANHTSCR CMSKLDVYRQ VHSIIRRSLP ATLPQCQAAN KTCPTNYMWN   250
H24828      ---------- ---------- ---------- ---------- ----------   250

HSVEGFCC    NHICRCLAQE DFMFSSDAGD DSTDGFHDIC GPNKELDEET CQCVCRAGLR   300
H24828      ---------- ---------- ---------- ---------- -------HLQE   300

HSVEGFCC    PASCGPHKEL DRNSCQCVCK NKLFPSQCGA NREFDENTCQ CVCKRTCPRN   350
H24828      PALCGPHMMF DEDRCECVCK TPCPKDLIQH PKNCSCFECK ESLETCCQKH   350

HSVEGFCC    QPLNPGKCAC ECTESPQKCL LKGKKFHHQT CSCYRRPCTN RQKACEPGF    400
H24828      KLFHPDTCSC E--------- --------DR CPFHTRPCAS GKTACAKHCR   400

HSVEGFCC    SYSEEVCRCV PSYWKRPQMS .......... .......... ..........   450
H24828      FPKEKRAAQG PHSRKNP... .......... .......... ..........   450
```

*HSVEGFCC: human VEGF-C

Fig. 3

```
HSVEGF-D     MYREWVVVNV FMMLYVQLVQ GSSNEHGPVK ---------- ------RSSQ     50
HSVEGF-C     MHLLGFFSVA CSLLAAALLP GPREAPAAAA AFESGLDLSD AEPDAGEATA     50
HSPDGF-A     MRTLACLLLL GCGYLAHVLA EEAEIPREVI ERLAR----- --------SQ     50
HSPDGF-B     MNRCWALFLS LCCYLRLVSA EGDPIPEELY EMLSD----- --------HS     50
HSPIGF2      MPVMRLFPCF LQLLAGLALP AVPPQQWALS AGNGS----- ----------     50
HSVEGF       MNFLLSWVHW SLALLLYLHH AKWSQAAPMA EGGGQ----- ----------     50
HSVEGF-B     MSPLLRR--- --LLLAALLQ LAPAQAPVSQ PDAPG----- ----------     50

HSVEGF-D     STLERSEQQI RAASSLEELL RITHSEDWKL WRCRLRLKSF TSMDSRSASH    100
HSVEGF-C     YASKDLEEQL RSVSSVDELM TVLYPEYWKM YKCQLRKGGW QHNREQANLN    100
HSPDGF-A     IHSIRDLQRL LEIDSVGSED S-L------- ---------- ----DTSLRA    100
HSPDGF-B     IRSFDDLQRL LHGDPGEEDG AEL------- ---------- ----DLNMTR    100
HSPIGF2      ---------- ---------- ---------- ---------- ----------    100
HSVEGF       ---------- ---------- ---------- ---------- ----------    100
HSVEGF-B     ---------- ---------- ---------- ---------- ----------    100

HSVEGF-D     RST----RFA ATFYDIETLK VIDEEWQRTQ CSPRETCVEV ASELGKSTNT    150
HSVEGF-C     SRTEETIKFA AAHYNTEILK SIDNEWRKTQ CMPREVCIDV GKEFGVATNT    150
HSPDGF-A     HGVHATKHVP EKRPLPIRRK RSIEEAVPAV CKTRTVIYEI PRSQVDPTSA    150
HSPDGF-B     SHSGGELESL ARGRRSLGSL TIAEPAMIAE CKTRTEVFEI SRRLIDRTNA    150
HSPIGF2      ---------- ---SEVEVVP FQEV-WGRSY CRALERLVDV VSEYPSEVEH    150
HSVEGF       ---------- ---NHHEVVK FMDV-YQRSY CHPIETLVDI FQEYPDEIEY    150
HSVEGF-B     ---------- ---HQRKVVS WIDV-YTRAT CQPREVVVPL TVELMGTVAK    150

HSVEGF-D     FF--KPPCVN VFRCGGCCNE ESLICMNTST SYISKQLFEI -SVPLTSVPE    200
HSVEGF-C     FF--KPPCVS VYRCGGCCNS EGLQCMNTST SYLSKTLFEI -TVPLSQGPK    200
HSPDGF-A     NFLIWPPCVE VKRCTGCCNT SSVKCQPSRV HHRSVKVAKV EYVRKKPKLK    200
HSPDGF-B     NFLVWPPCVE VQRCSGCCNN RNVQCRPTQV QLRPVQVRKI EIVRKKPIFK    200
HSPIGF2      MF--SPSCVS LLRCTGCCGD ENLHCVPVET ANVTMQLLKI ---RSGDRPS    200
HSVEGF       IF--KPSCVP LMRCGGCCND EGLECVPTEE SNITMQIMRI ---KPHQGQH    200
HSVEGF-B     QL--VPSCVT VQRCGGCCPD DGLECVPTGQ HQVRMQILMI ---RYPSSQ-    200

HSVEGF-D     LVPVKVANHT GCKCLPT--A PRHPYSIIRR SIQIPEEDRC SHSKKLCPID    250
HSVEGF-C     PVTISFANHT SCRCMSKLDV YRQVHSIIRR S-LPATLPQC QAANKTCPTN    250
HSPDGF-A     EVQVRLEEHL ECACATTSLN PDYREEDTGR P-RESGKKRK R--KRLKPT.    250
HSPDGF-B     KATVTLEDHL ACKCETVAAA ARPVTRSPGG S-QEQRAK-- ----------    250
HSPIGF2      YVELTFSQHV RCECRP---- LREKMKPERC R-PKGRGKRR R--EKQRPT-    250
HSVEGF       IGEMSFLQHN KCECRP-KKD RARQEKKSVR G-KGKGQKRK R--KKSRYK-    250
HSVEGF-B     LGEMSLEEHS QCECRPKKKD SA-------- ---------- ----------    250

HSVEGF-D     MLWDSNKCKC VLQEE-NPLA GTEDHSHLQE ---------- ----------    300
HSVEGF-C     YMWNNHICRC LAQEDFMFSS DAGDDSTDGF HDICGPNKEL DEETCQCVCR    300
HSPDGF-A     .......... .......... .......... .......... ..........    300
HSPDGF-B     ---------- ---------- ---------- ---------- TPQTRVTIRT    300
HSPIGF2      ---------- ---------- --DCHLCGDA VPRR...... ..........    300
HSVEGF       ----SWSVYV GARCCLMPWS LPGPHPCGPC SERRKHLFVQ DPQTCKCSCK    300
HSVEGF-B     ---------- ---------- VKPDSPRPLC PRCTQHHQRP DPRTCRCRCR    300

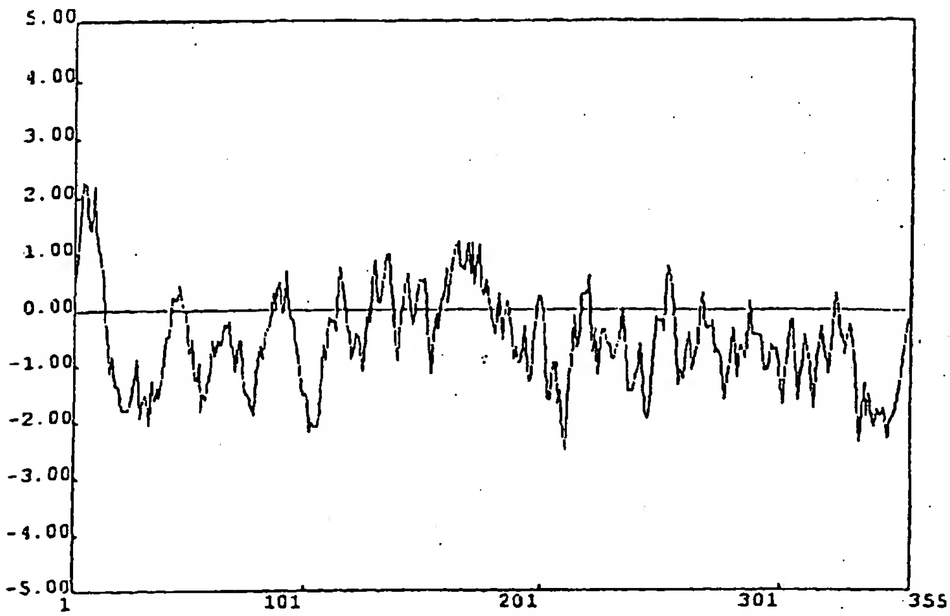
HSVEGF-D     ----PALCGP HMMFDEDRCE CVCKTPCPKD LIQHPKNCSC FECKESL-ET    350
HSVEGF-C     AGLRPASCGP HKELDRNSCQ CVCKNKLFPS QCGANREFDE NTCQCVCKRT    350
HSPDGF-A     .......... .......... .......... .......... ..........    350
HSPDGF-B     VRVRRPPKGK HRKFKHTHDK TALKETLGA. .......... ..........    350
HSPIGF2      .......... .......... .......... .......... ..........    350
HSVEGF       N-TDSRCKAR QLELNERTCR CDKPRR.... .......... ..........    350
HSVEGF-B     RRSFLRCQGR GLELNPDTCR CRKLRR.... .......... ..........    350

HSVEGF-D     CCQKHKLFHP DTCSCE---- ---------- ---DRCPFHT RPCASGKTAC    400
HSVEGF-C     CPRNQPL-NP GKCACECTES PQKCLLKGKK FHHQTCSCYR RPCTNRQKAC    400
HSPDGF-A     .......... .......... .......... .......... ..........    400
HSPDGF-B     .......... .......... .......... .......... ..........    400
HSPIGF2      .......... .......... .......... .......... ..........    400
HSVEGF       .......... .......... .......... .......... ..........    400
HSVEGF-B     .......... .......... .......... .......... ..........    400

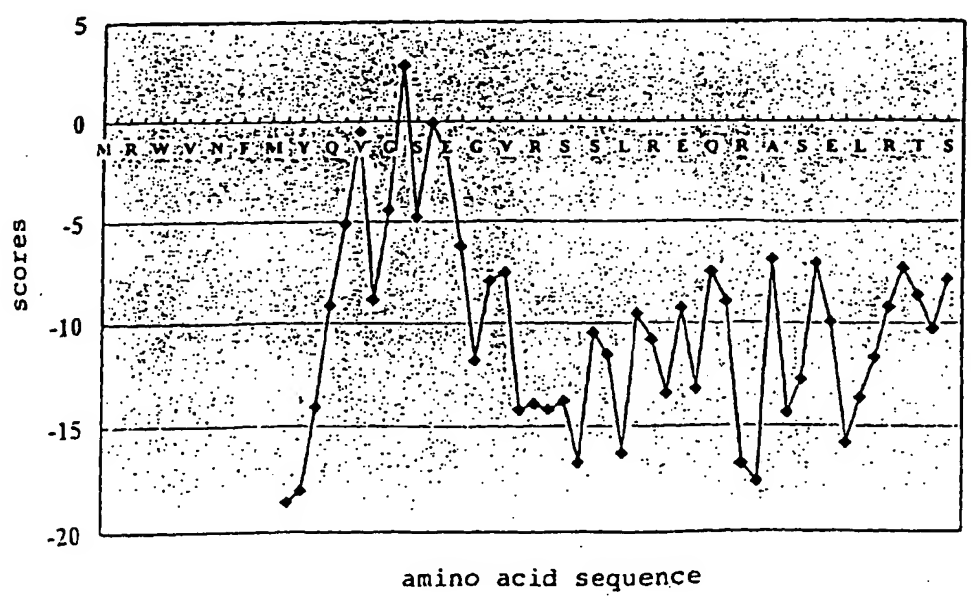
HSVEGF-D     AKHCRFPKEK RAAQGPHSRK NP........ .......... ..........    450
HSVEGF-C     -EPGFSYSEE VCRCVPSYWK RPQMS..... .......... ..........    450
HSPDGF-A     .......... .......... .......... .......... ..........    450
HSPDGF-B     .......... .......... .......... .......... ..........    450
HSPIGF2      .......... .......... .......... .......... ..........    450
HSVEGF       .......... .......... .......... .......... ..........    450
HSVEGF-B     .......... .......... .......... .......... ..........    450
```

Fig. 4

a) Hydrophobicity



b) Prediction of the human VEGF-D signal peptide



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/02456

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl[6] C12N15/18, C12N15/63, C12P21/02, C07K14/485, C07K16/22, G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl[6] C12N15/18, C12N15/63, C12P21/02, C07K14/485, C07K16/22, G01N33/50

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, WPI/L, BIOSIS PREVIEWS, CAS ONLINE, GENETYX-MAC/CD

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	Yamada, Y. et al. "Molecular cloning of a novel vascular endothelial growth factor, VEGF-D." Genomics (1997, Jun.), Vol. 42, No. 3, p. 483-488	1 - 10
X	Vladimir, J. et al. "A novel vascular endothelial growth factor, VEGF-C, (VEGFR-2) receptor tyrosine kinases" EMBO J. (1996, Jan.) Vol. 15, No. 2, p. 290-298	1 - 2
X	Vladimir, J. et al. "A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4(VEGFR-3) and KDR(VEGFR-2) receptor tyrosine kinases" EMBO J. (1996, Jan.) Vol. 15, No. 7, p. 1751	1 - 2
PX	Maurizio, O. et al. "Identification of a c-fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family" Proc. Natl. Acad. Sci. USA (1996, Oct.) Vol. 93, p. 11675-11680	1 - 2

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
October 7, 1997 (07. 10. 97)	October 21, 1997 (21. 10. 97)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/02456

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Georg. B. et al. "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation" Development (1992) Vol. 114, p. 521-532	1 - 10
X	David, T.S. et al. "The mouse gene for vascular endothelial growth factor" J. Biol. Chem. (1996, Feb.) Vol. 271, No. 7, p. 3877-3883	1 - 10
X	Kevin, P.C. et al. "Vascular endothelial growth factor" J. Biol. Chem. (1992) Vol. 267, No. 23, p. 16317-16322	1 - 10
X	Greg, C. et al. "Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor" Proc. Natl. Acad. Sci. USA (1990) Vol. 87, p. 2628-2632	1 - 10
X	Edmund, T. et al. "The human gene for vascular endothelial growth factor" J. Biol. Chem. (1991) Vol. 266, No. 18, p. 11947-11954	1 - 10

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/02456

Disclosure other than written disclosures

1. The GenBank Database (Rel. 100) on GENETYX, Accession No. D89628, Yoshiki Yamada, Chugai Research Institute for Molecular Medicine. (29-Nov-1996)

2. The GenBank Database (Rel. 100) on GENETYX, Accession No. T64277, Hillier, L. et al. (1995)